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Inactivation of Bacillus Anthracis Spores Delivered as Liquid Suspension or Aerosol to Self-Decontaminating Fabric

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Air Force Research Laboratory
Human Effectiveness Directorate
Biosciences and Protection Division
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Aberdeen Proving Ground MD

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Inactivation of *Bacillus anthracis* Spores Delivered as Liquid Suspension or Aerosol to Self-Decontaminating Fabric

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BACKGROUND: Military fabric amended with an antimicrobial compound could reduce the viability of biological agents that could be encountered during operations in contaminated environments. In this study, military fabric treated with a chlorine-based compound was evaluated for activity against the *Bacillus anthracis* spore delivered as an aerosol or a liquid suspension. **METHODS:** Military fabric samples with and without antimicrobial treatment were inoculated with *B. anthracis* spores from an aqueous suspension and incubated in an exposure chamber under controlled relative humidity (RH) and temperature. In addition, a stream of aerosolized *B. anthracis* spores was delivered to fabric samples under controlled conditions. After specified time intervals of exposure in the chamber or the aerosol system, spores were eluted from fabric samples and enumerated by cultivation on Nutrient Agar and direct microscopic count. Efficacy of the chlorine-based compound was assessed by comparing cultivable percentages of spores eluted from the treated fabric to cultivable percentages of spores eluted from untreated fabrics or treated fabrics at the initial exposure time. **RESULTS:** When spores were delivered to fabric as an aqueous suspension and incubated in the exposure chamber at 30°C with greater than 90% RH, cultivability was reduced by greater than two logarithms after 1 hour and from four to six logarithms after 2 hours. When spores were delivered as an aqueous suspension and incubated in the chamber for up to 24 hours at 30°C with 20% RH, cultivability was reduced by less than one logarithm. Spores delivered to fabric as an aerosol for one or two hours appeared not to be affected by the antimicrobial. However, spores delivered as an aerosol to treated fabric were inactivated upon subsequent incubation in the exposure chamber at greater than 90% RH. **CONCLUSIONS:** *B. anthracis* spores can be killed during contact with military fabric amended with a chlorine-based compound. However, temperature and relative humidity are factors in the degree of inactivation.

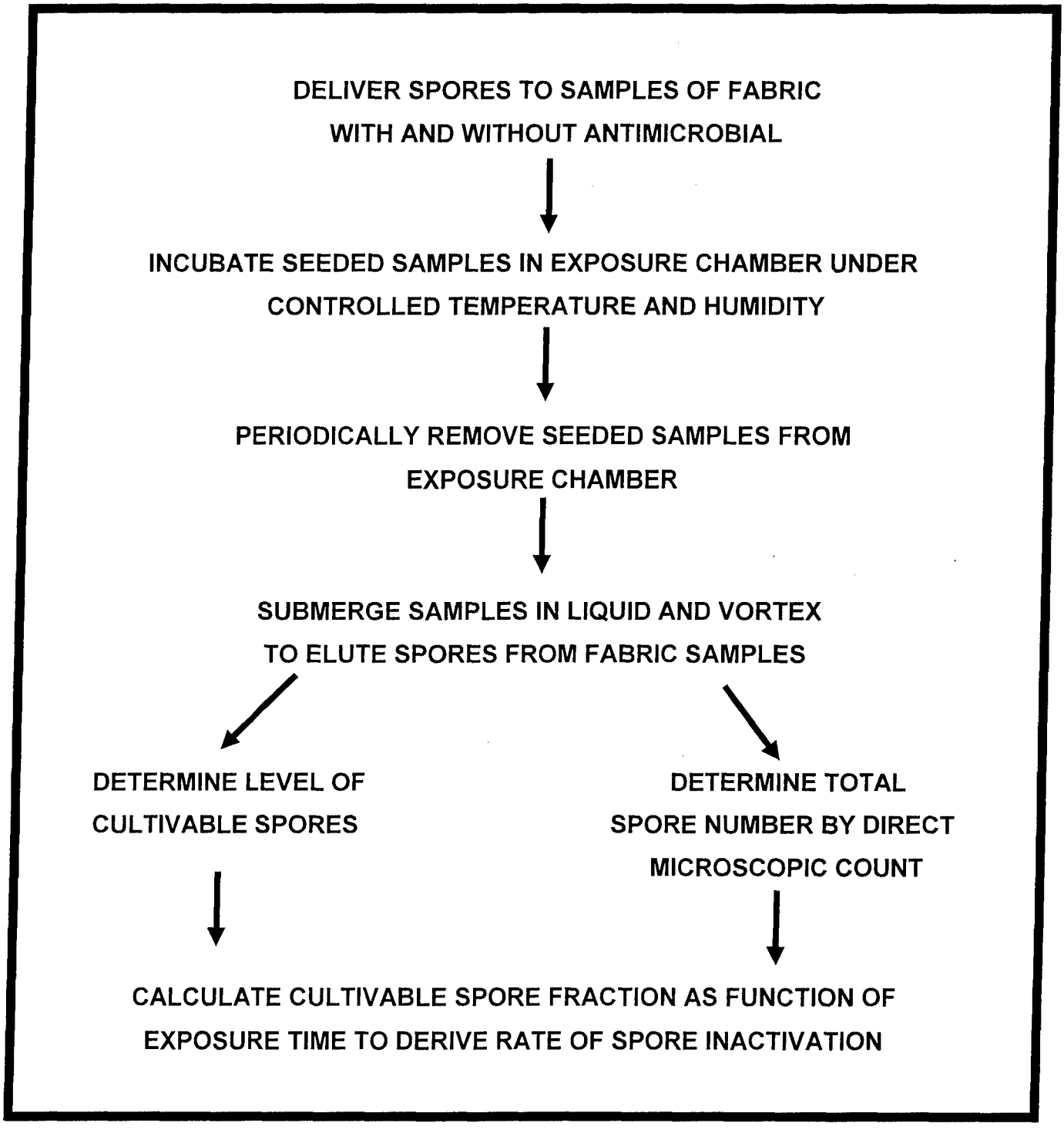
DELIVER SPORES TO SAMPLES OF FABRIC WITH AND WITHOUT ANTIMICROBIAL
INCUBATE SEEDED SAMPLES IN EXPOSURE CHAMBER UNDER CONTROLLED TEMPERATURE AND HUMIDITY
PERIODICALLY REMOVE SEEDED SAMPLES FROM EXPOSURE CHAMBER
SUBMERGE SAMPLES IN LIQUID AND VORTEX TO ELUTE SPORES FROM FABRIC SAMPLES
DETERMINE LEVEL OF CULTIVABLE SPORES
DETERMINE TOTAL SPORE NUMBER BY DIRECT MICROSCOPIC COUNT
CALCULATE CULTIVABLE SPORE FRACTION AS FUNCTION OF EXPOSURE TIME TO DERIVE RATE OF SPORE INACTIVATION

FIG 1

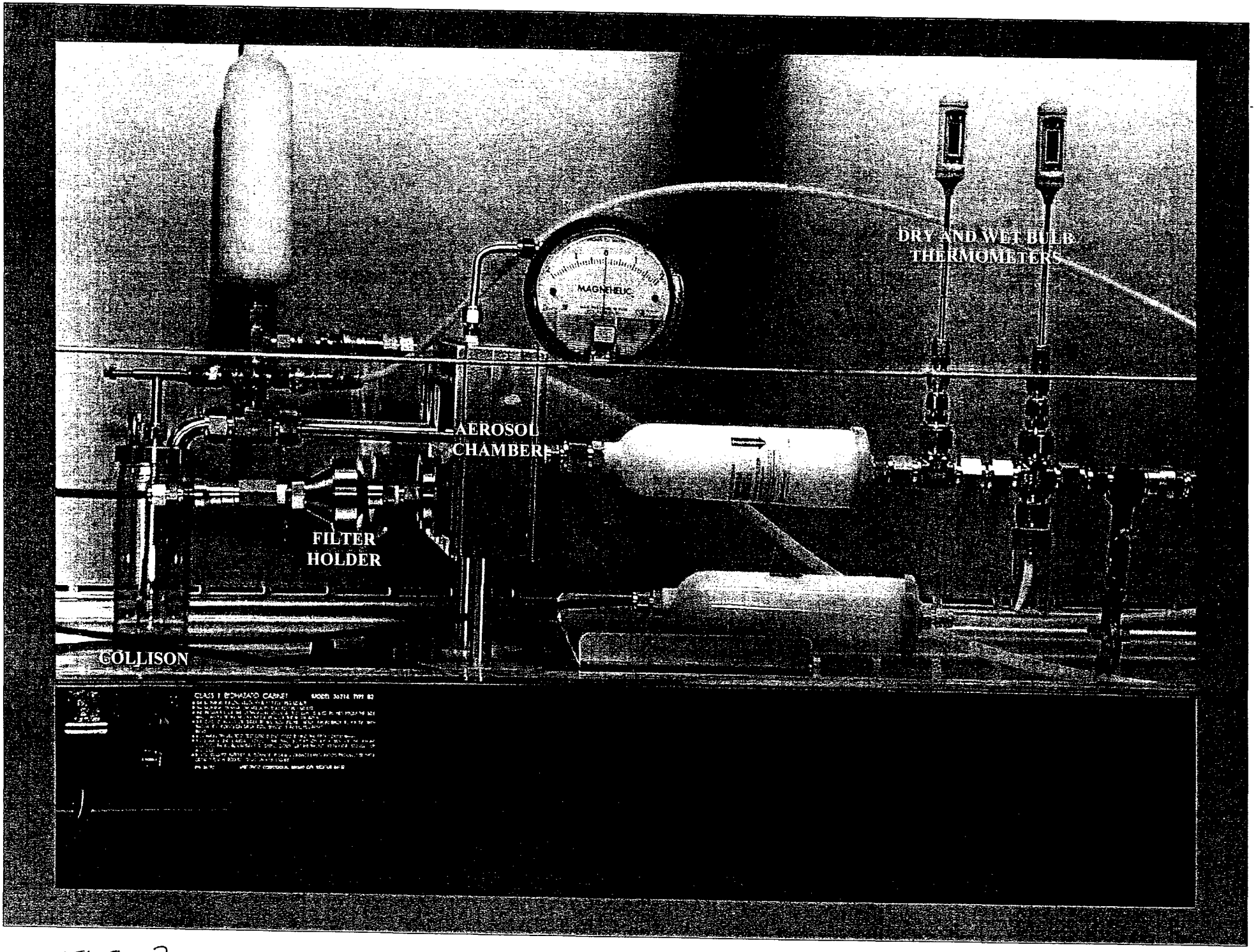
DRY AND WET BULB
THERMOMETERS
MAGNEHELIC
AEROSOL
CHAMBER
FILTER
HOLDER
COLLISON

FIG 2

AEROSOL TEST SYSTEM (ATS) SCHEMATIC

- Multiport chamber for side-by-side filter comparisons
- Mass flow controller for each filter unit for controlled aerosol particle delivery
- Controlled humidity for dry or humid filter trials

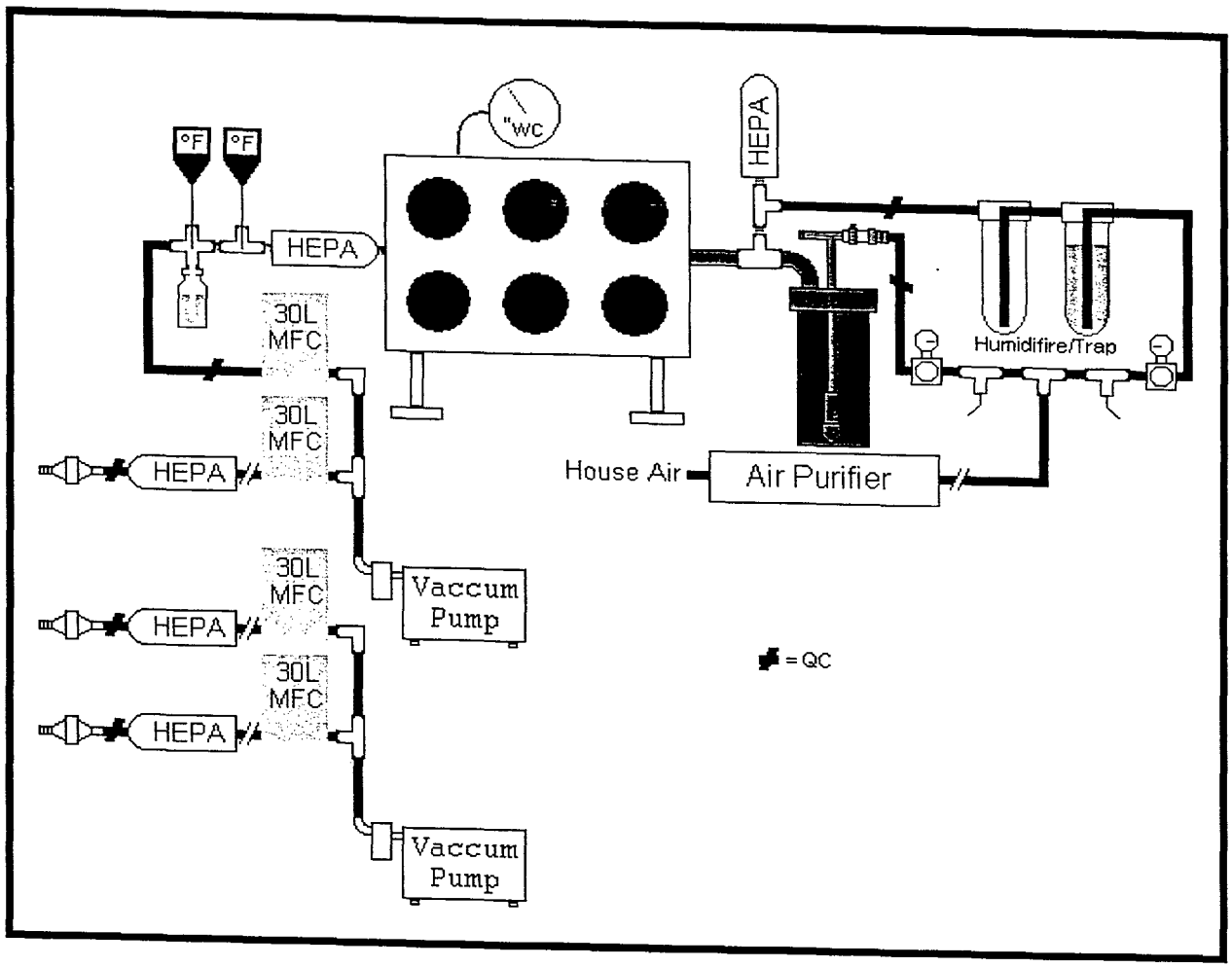

FIG 3

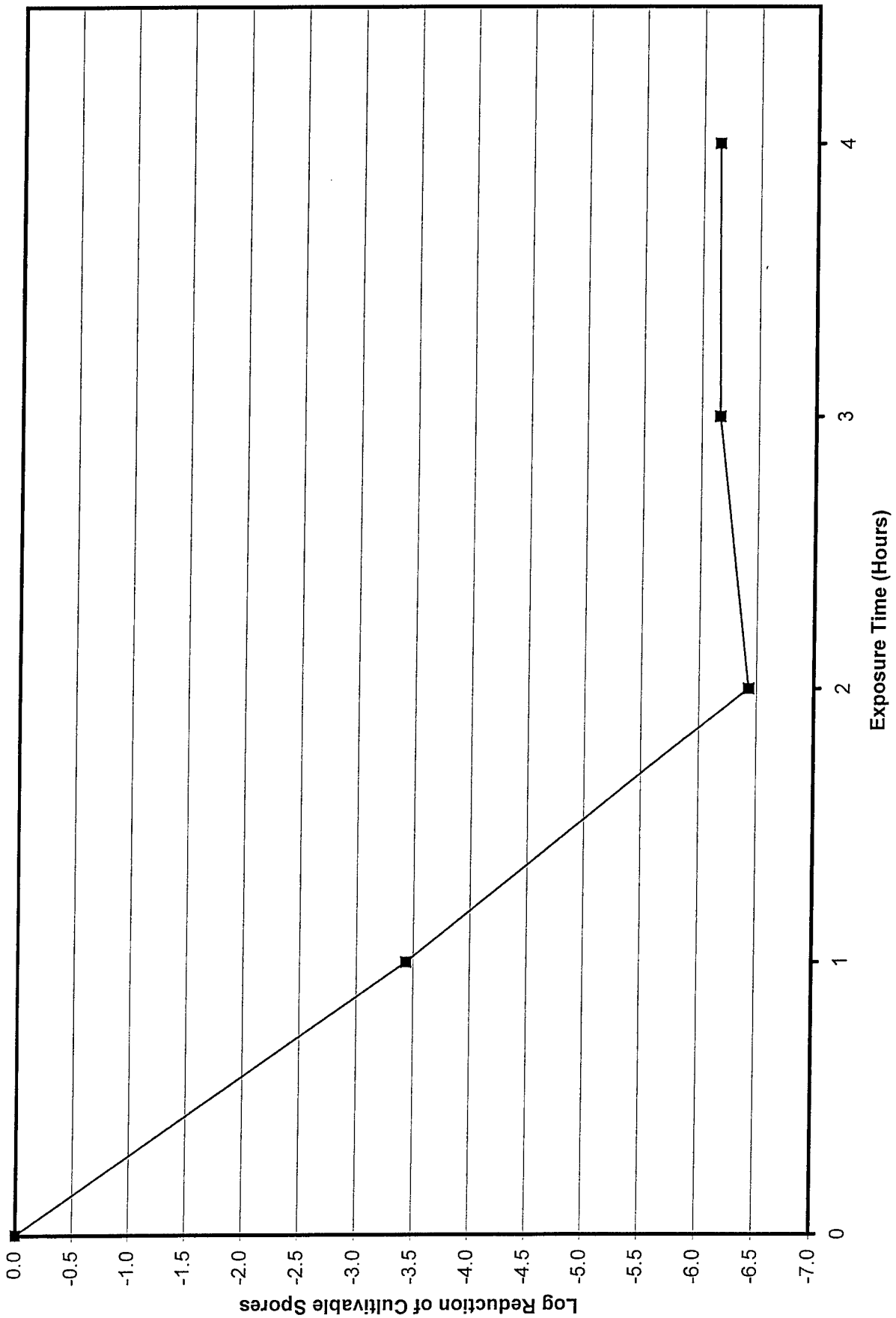
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
-4.0
-4.5
-5.0
-5.5
-6.0
-6.5
-7.0
Log Reduction of Cultivable Spores
0
1
2
3
4
Exposure Time (Hours)

FIG 4

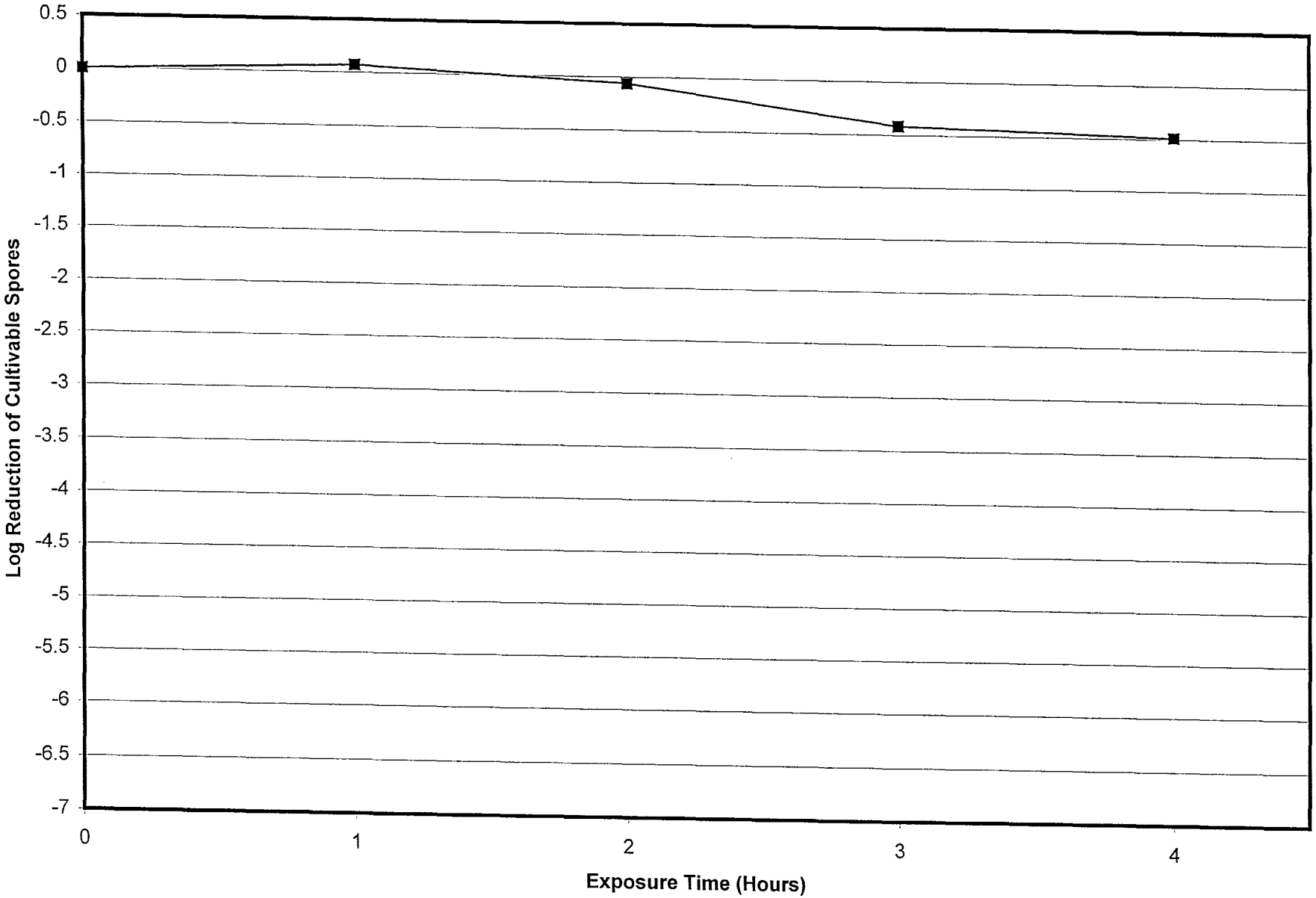
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5
-6
-6.5
-7
Log Reduction of Cultivable Spores
0
1
2
3
4
Exposure Time (Hours)

FIG 5

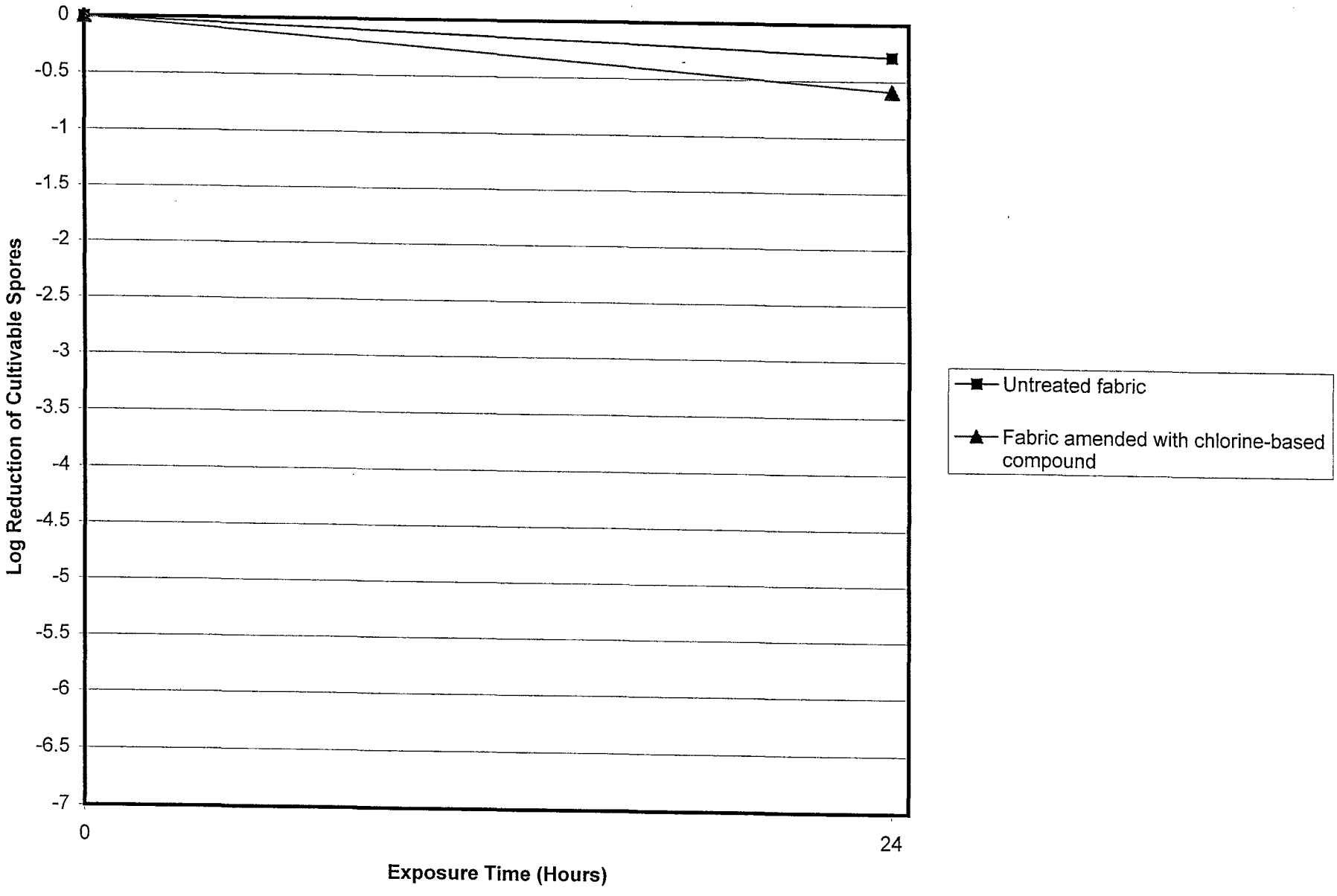
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5
-6
-6.5
-7
Log Reduction of Cultivable Spores
0
24
Exposure Time (Hours)
Untreated fabric
Fabric amended with chlorine-based compound

FIG 6

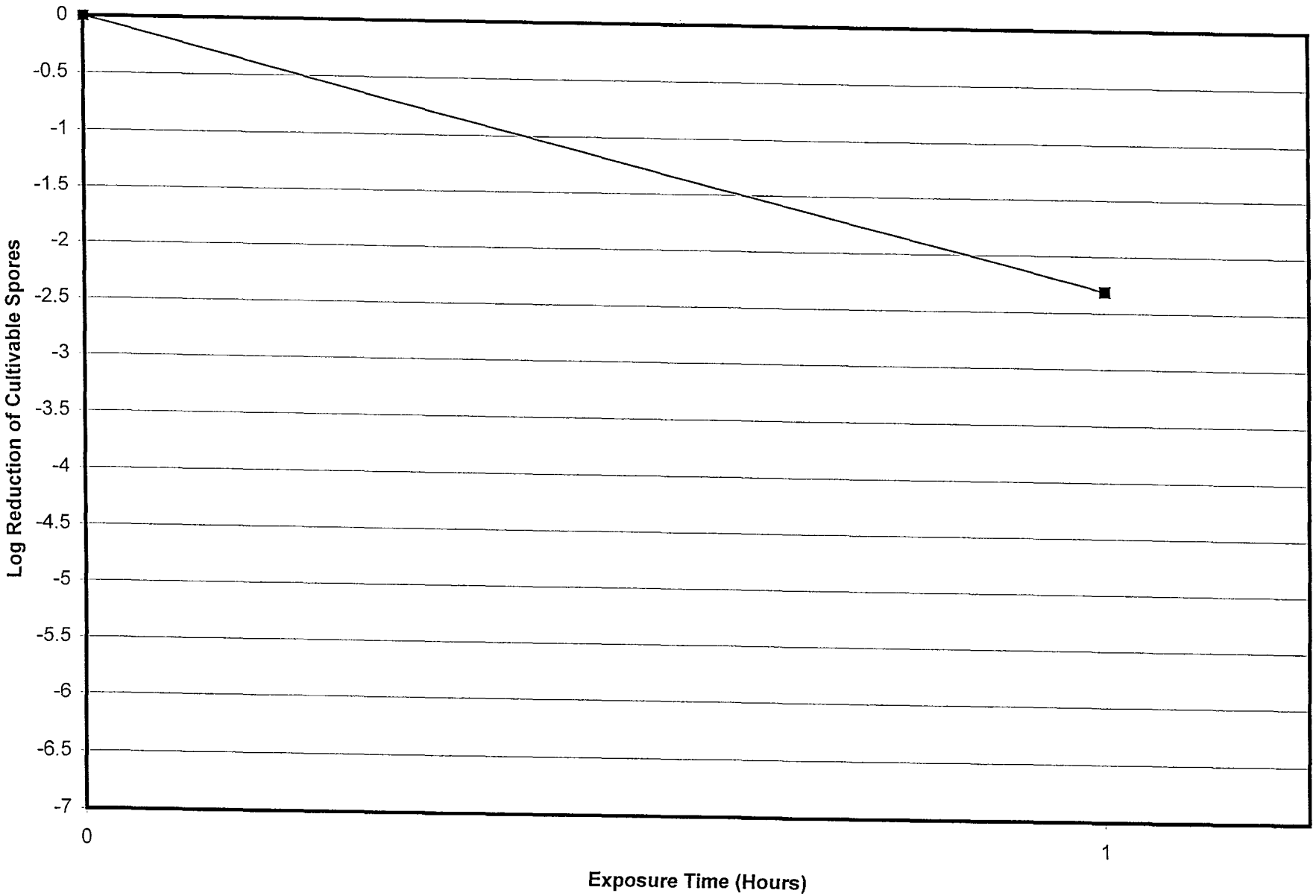
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5
-6
-6.5
-7
Log Reduction of Cultivable Spores
0
1
Exposure Time (Hours)

FIG 7

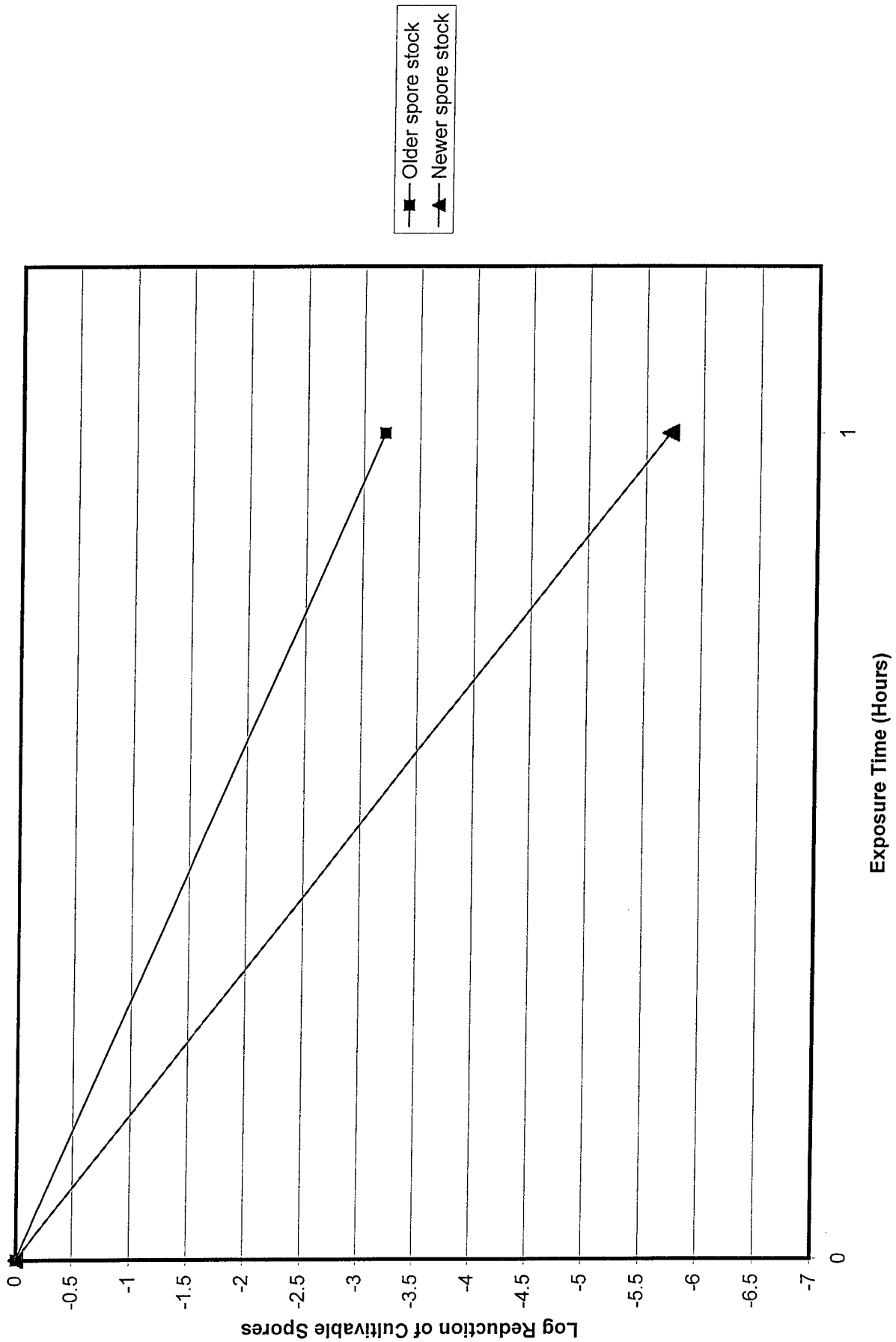
Older spore stock
Newer spore stock
Log Reduction of Cultivable Spores
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5
-6
-6.5
-7
0
1
Exposure Time (Hours)

FIG 8

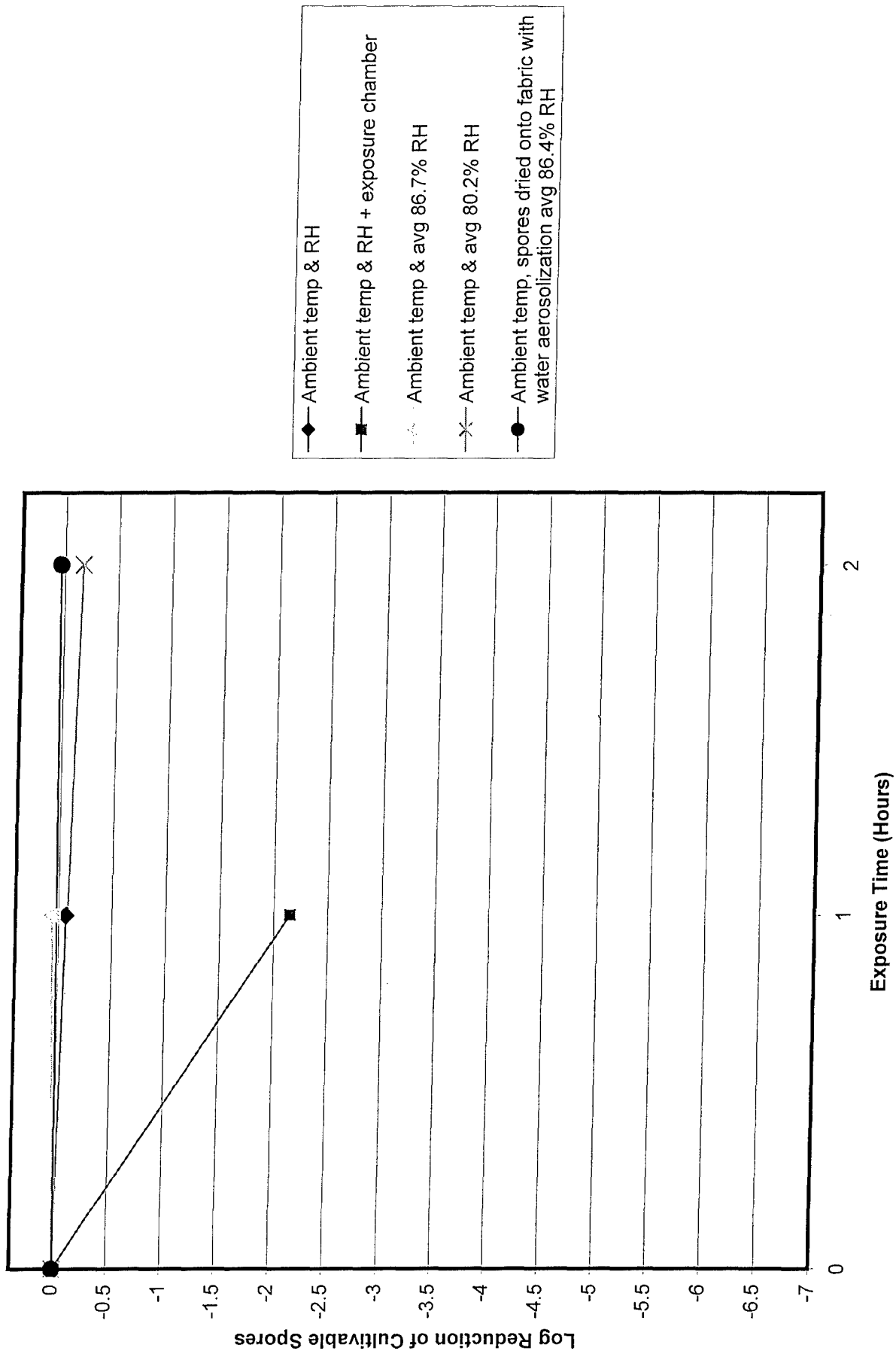
Ambient temp & RH
Ambient temp & RH + exposure chamber
Ambient temp & avg 86.7% RH
Ambient temp & avg 80.2% RH
Ambient temp, spores dried onto fabric with water aerosolization avg 86.4% RH
Log Reduction of Cultivable Spores
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5
-6
-6.5
-7
0
1
2
Exposure Time (Hours)

FIG 9

FIG 1. Schematic of testing protocols to assess efficacy of antimicrobial compound for killing *B. anthracis* spores on military fabric. *B. anthracis* spores in aqueous suspension were delivered to circular samples of untreated fabric and fabric amended with a chlorine-based compound. After specified times of exposure in a chamber with equilibrated temperature and relative humidity, spores were eluted from fabric by vortex in liquid. Eluted spores were evaluated by direct microscopic count with a Petroff-Hausser counting chamber and cultivability was determined by membrane filtration and Nutrient Agar plate counts. Percent cultivability was based on the ratio of cultivable spores to total spores. Efficacy was based on the rate of spore inactivation as compared with spores delivered to untreated fabric or at the initial exposure time.

FIG 2. Image of the Aerosol Test System (ATS) employed for laboratory testing. The ATS was established to generate and deliver aerosolized microorganism to filters under controlled conditions. The ATS is being used to test fabric amended with a chlorine-based compound for inactivating *B. anthracis* spores delivered to the fabric as an aerosol.

FIG 3. Schematic of the ATS employed for exposing aerosolized spores to materials amended with antimicrobial compounds. The ATS employs a Collison nebulizer to produce aerosols that are delivered to a multi-port chamber. Aerosol relative humidity is adjusted by introducing humidified air to the line between the nebulizer and aerosol chamber. Relative humidity is monitored using dry-bulb and wet-bulb thermometers located in a line exiting the chamber. The aerosol chamber has six ports for material holders. Each port used in a trial has its own mass flow controller to regulate and monitor air flow. The multi-port system allows side-by-side comparisons of untreated and antimicrobial-treated materials.

FIG 4. Log reduction of cultivable *B. anthracis* spores over four hours in exposure chamber at 30°C and greater than 90% RH. Spores were delivered in aqueous suspension to fabric amended with a chlorine-based compound and eluted by vortex in liquid after 0, 1, 2, 3, and 4 hours of exposure to 30°C and greater than 90% RH.

FIG 5. Log reduction of cultivable *B. anthracis* spores over four hours in exposure chamber at 30°C and about 20% RH. Spores were delivered in aqueous suspension to fabric amended with a chlorine-based compound and eluted by vortex in liquid after 0, 1, 2, 3, and 4 hours of exposure to 30°C and about 20% RH.

FIG 6. Log reduction of cultivable *B. anthracis* spores over twenty-four hours in exposure chamber at 30°C and about 20% RH. Spores were delivered in aqueous suspension to fabric amended with a chlorine-based compound and eluted by vortex in liquid after 0 and 24 hours of exposure to 30°C and about 20% RH.

FIG 7. Average log reduction of cultivable *B. anthracis* spores over one hour in exposure chamber at 30°C and greater than 90% RH over three trials. Spores were delivered in aqueous suspension to fabric amended with a chlorine-based compound and eluted by vortex in liquid after 1 hour of exposure to 30°C and greater than 90% RH. Fabric exhibited the same spore inactivation levels after a regular cycle cold water wash

in a domestic washing machine as before washing, demonstrating that the compound is stable.

FIG 8. PRELIMINARY Average log reduction of cultivable *B. anthracis* spores over one hour in exposure chamber at 30°C and about 80% RH over three trials. Spores were delivered in aqueous suspension to fabric amended with a chlorine-based compound and eluted by vortex in liquid after 1 hour of exposure to 30°C and about 80% RH. *The older spore stock exhibits less reduction in cultivability than the newer spore stock on the same fabric. We are currently investigating age of originating spores for culture as the cause.*

FIG 9. PRELIMINARY Log reduction of cultivable *B. anthracis* spores using the ATS. Reduction of spore cultivability is not seen at ambient temperature, ambient relative humidity or, increased relative humidity (greater than 80%). Reduction of spore cultivability was only seen when fabrics were incubated in exposure chamber at 30°C and greater than 90% RH. *Further trials are being done with this system.*

SUMMARY

- *Bacillus anthracis* spore cultivability was reduced by greater than two logarithms when spores were delivered as a aqueous suspension to a military fabric treated with a chlorine-based compound and incubated in an exposure chamber for 1 hour at 30°C at greater than 90% relative humidity.

- *B. anthracis* spore cultivability was reduced by greater than six logarithms when spores were delivered as an aqueous suspension to a military fabric treated with a chlorine-based compound and incubated in an exposure chamber for 2 hours at 30°C at greater than 90% relative humidity.

- *B. anthracis* spore cultivability was reduced by less than one logarithm when spores were delivered as an aqueous suspension to military fabric treated with a chlorine-based compound and incubated in an exposure chamber for up to 24 hours at 30°C at approximately 20% relative humidity.

- *B. anthracis* spore cultivability was reduced by two to six logarithms when spores were delivered as an aqueous suspension to military fabric treated with a chlorine-based compound and incubated in an exposure chamber for 1 hour at 30°C at approximately 80% relative humidity. Further experiments are being completed to determine if age of spore stock is responsible for the range of reduction seen.

- Preliminary experiments suggested that there was no significant reduction in cultivability when *B. anthracis* spores were delivered as an aerosol to military fabric treated with a chlorine-based compound with or without humidity in the aerosol test chamber. However, spore cultivability was a reduced by greater than two logarithms when spores were delivered as an aerosol to the fabric at ambient temperature and humidity followed by fabric incubation in the exposure chamber for 1 hour at 30°C at greater than 90% relative humidity.